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(54) Title: A BINDING MOTIF AND METHODS OF REGULATING CELL FUNCTION

(57) Abstract: The present invention relates to a binding motif and methods of regulating cell function which methods target a single amino acid residue preferably a Tyr in a binding motif equivalent to a motif of the common beta chain (ßc) of the GM-CSF/IL-3/IL-5 receptor. Preferably, the cell functions affect cell survival and proliferation in cells. The methods can be used for treatments of conditions relating to cell survival and proliferation and can be used to expand progenitor cells, for instance, for transplantation purposes.

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A BINDING MOTIF AND METHODS OF REGULATING CELL FUNCTION

FIELD OF THE INVENTION

The present invention relates to a binding motif and methods of regulating cell function which methods target a single amino acid residue in a binding motif equivalent to a motif of the common beta chain (βc) of the GM-CSF/IL-3/IL-5 receptor. Preferably, the cell functions affect cell survival and proliferation in cells. The methods can be used for treatments of conditions relating to cell survival and proliferation and can be used to expand progenitor cells, for instance, for transplantation purposes.

BACKGROUND

The number of haematopoietic cells generated *in vitro* and *in vivo* is tightly regulated by the integration of survival, proliferation and differentiation signals that emanate from growth factor receptors. Defining the molecular mechanisms regulating these processes is critical for the design of new strategies to expand haematopoietic progenitor cells and their progeny for bone marrow transplantation, and for our understanding of leukaemia, myeloproliferative diseases and chronic inflammation where the normal balance of cell production and function has broken down.

Although many cytokines such as IL-3, GM-CSF and IL-5 and growth factors such as PDGF and IGF-1 were initially discovered as mitogens by virtue of their ability to promote cell proliferation, many of these factors were later also found to be potent regulators of cell survival through their ability to suppress programmed cell death or apoptosis. These biological activities are regulated by the binding of the cytokine or growth factor to its cognate cell surface receptor which initiates an ordered series of signalling events that includes receptor dimerization, the activation of tyrosine kinases followed by the tyrosine phosphorylation of the receptor cytoplasmic tail, the binding of multiprotein signalling complexes to receptor phosphotyrosine residues via src-homology 2 (SH2) domains or phosphotyrosine-binding (PTB) domains and the activation of downstream signalling cascades that promote a cellular response.

Many factors including cytokines can contribute to cell survival and proliferation and the regulation of these to easily manipulate control is not a simple matter. Identification of one controlling factor can assist in the development of useful treatments and diagnosis of conditions where cell production and function are out of balance.

The action of signalling molecules such as cytokines has been poorly understood. It is apparent that these cellular proteins can switch on activities within cells. However, the actual triggering mechanisms and how these are relayed to culminate in their final activities is not known. Cell cycles are clearly involved but the link between the signalling molecule and receptor and actions such as cell survival, proliferation, and differentiation is unclear.

Proteins including human granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5 are capable of stimulating normal and transformed hematopoietic cells. With each, the initiating event for signal transduction is the binding of the protein to its surface receptors. These receptors may be composed of subunits such as the α chain and a common β chain (β_c). Engagement of β_c by the binding of the cytoplasmic protein to surface receptors results in the stimulation of cell survival, proliferation, and differentiation and mature cell effector function in the appropriate lineage, a fact that emphasises the major signalling role played by β_c in mediating receptor induced biological activities.

One of the first events in activation of receptors and in the initiation of the signalling cascade is tyrosine phosphorylation of β_c. This is a common theme among receptor signalling subunits and can be seen in homodimeric receptors such as the erythropoietin (EPO) receptor, thrombopoietin (TPO) receptor, and granulocyte colony-stimulating factor (G-CSF) receptor as well as in heterodimeric receptors such as in the IL-6 and IL-2 receptors, and in the GM-CSF, IL-3, and IL-5 receptor systems.

Tyrosine phosphorylation of receptor signalling subunits appeared to be a critical step in the creation of docking sites for the association of signalling molecules. Despite the perceived importance of tyrosine phosphorylation of receptors it is becoming apparent in some cells that signalling can proceed in its absence. This is demonstrated in the EPO and TPO receptors, in which the substitution of all tyrosines failed to abolish their activities.

It has been unclear until now how the binding of proteins to their receptors can result in the specialised functions associated with these molecules and their receptors.

SUMMARY OF THE INVENTION

In a first aspect of the present invention, there is provided a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine or an equivalent thereof.

A single amino acid corresponding to Tyr577 of βc of the GM-CSF receptor has been identified as a controlling factor in the regulation of cellular activities. For this reason, this invention targets this amino acid residue for modulating cellular activity associated with the GM-CSF receptor of the GM-CSF cytokine.

In a preferred embodiment, there is provided a binding motif of a receptor. The receptor may be any receptor that is capable of binding to an extracellular molecule or protein and which mediates its function through the binding of a cytoplasmic molecule or protein such as Shc, or any cytoplasmic molecule or protein capable of binding a further signalling molecule which activates a cascade of events leading to cell signalling pathways and biological functions such as mitogenesis, proliferation, transformation, differentiation and cell survival, or any other cytoplasmic molecule or protein.

In another aspect of the present invention there is provided a method of modulating activity in a cell, said method including:

introducing a modification to a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

In another aspect of the present invention there is provided a method modulating activity in a cell, said method including:

introducing a modification to a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine and wherein the tyrosine is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor or an equivalent thereof.

Accordingly, in yet another preferred embodiment, the invention provides a method of modulating activity in a cell, said method including

modifying phosphorylation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

In yet another preferred aspect of the present invention, there is provided a method of increasing cell growth, said method including

inhibiting activation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

In yet another aspect of the present invention there is provided a method of transplantation of cells or enhancing transplantation efficiency, said method including

inhibiting activation in one or more cells of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following

N-X-X-Y

wherein X is any residue, and Y is tyrosine; and

transplanting the cells into a patient in need of such treatment.

In another aspect of the present invention there is provided a method of improving wound healing in a patient, said method including

inhibiting activation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine in a region of the wound.

In yet another aspect of the present invention, there is provided a method for screening cell growth promoting compounds, said method including

providing a cell in which phosphorylation of the Tyr577 or an equivalent has been induced;

exposing the cell to the compound; and

assessing colony formation of the cells.

FIGURES

Figure 1 shows Y577F mutation leads to enhanced numbers of colonies in response to GM-CSF.

Figure 2 shows Y577F point mutation can give rise to larger colonies than wild type GM-CSF receptor beta chain.

Figure 3 shows foetal liver cells transduced with Y577F mutant of the beta chain form greater numbers of colonies at all concentrations of GM-CSF.

Figure 4a shows a Delta Assay of cells cultured for 7 days prior to colony formation.

Figure 4b shows a Delta Assay of cells cultured for 14 days prior to colony formation.

Figure 5 shows CTL cells expressing the human GM-CSF receptor have greater response to GM-CSF when Y577 is mutated to phenylalanine.

Figure 6 shows the amino acid sequence of βc.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect of the present invention, there is provided a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine or an equivalent thereof.

The term "motif" as used herein, means a distinctive amino acid sequence which is conserved and forms a unit in which the amino acids interact.

Signalling molecules may be molecules involved in cellular pathways such as but not limited to those pathways involved in proliferation, survival or differentiation. Examples of such pathways may include the JAK/STAT pathway, the ras/MAP kinase pathway or the PI-3-Kinase pathway. All pathways may be involved directly or indirectly with these functions.

The term "cell signalling pathways" as used herein includes all cellular pathways and cellular reactions which contribute to the functioning of the cell. It is not restricted to reactions that arise from cytokine mediated binding to the receptor. However, it is most preferred that the activities are activated by cytokine binding.

The cytoplasmic protein will be one that interacts with a cell receptor through a particular amino acid, namely tyrosine, however, it is preferred that the cytoplasmic proteins that bind to the amino acid are selected from the group including 14-3-3 protein, Shc, SHIP-2, WW-domain of the prolyl isomerase, Pin1 and the ubiquitin ligase, NEDD4 and any cytoplasmic protein capable of binding a further signalling molecule which activates a cascade of events

functions in a cell such as mytogenesis, proliferation, transformation, differentiation and cell survival. More preferably, the cytoplasmic protein is Shc. Most preferably, Shc will bind to tyrosine.

The term "signalling molecule" is any molecule that can signal an activation in the signalling pathway.

Shc will bind to Tyr via its PTB domain and has the potential to both positively and negatively regulate intracellular signalling. For example, in addition to its suggested positive role in promoting signalling via the Ras/Map kinase pathway through the recruitment of grb2/sos and via the PI 3-kinase pathway through the recruitment of a grb2/GAB2/PI 3-kinase complex, Shc is also known to recruit negative regulators of signalling including the phosphatases SHP2 and SHIP.

The cytoplasmic proteins which bind to the amino acid will in turn bind to further signalling molecules which can activate a cascade of events leading to cell signalling pathways and biological functions such as, but not limited to, mitogenesis, proliferation, transformation, differentiation and cell survival or any other cytoplasmic molecule or protein.

Preferably, the tyrosine residue can react with cytoplasmic proteins and in turn the tyrosine and cytoplasmic protein can interact to activate cellular activity .

More preferably, the cytoplasmic protein that binds to tyrosine is Shc, or SHIP-2.

In a preferred embodiment, there is provided a binding motif of a receptor. The receptor may be any receptor that is capable of binding to an extracellular molecule or protein and which mediates its function through the binding of a cytoplasmic molecule or protein such as Shc, or any cytoplasmic molecule or protein capable of binding a further signalling molecule which activates a cascade of events leading to cell signalling pathways and biological functions such as mitogenesis, proliferation, transformation, differentiation and cell

A receptor as used herein may be selected from the group including:

(1) GM-CSF/IL-3/IL-5 receptor

(2) IL6 human interleukin-6 receptor beta chain precursor (IL-6R-beta)

(3) LEPR human leptin receptor precursor (LEP-R) (OB RECEPTOR) (OB-R).

(4) TNR2 human tumor necrosis factor receptor 2 precursor (tumor necrosis factor

(5) VGR1 human vascular endothelial growth factor receptor 1 precursor

(6) TRK3 human receptor protein-tyrosine kinase TKT precursor (EC 2.7.1.112)

(7) Q01974 protein-tyrosine kinase transmembrane receptor ROR2 precursor

(8) FGR1 human basic fibroblast growth factor receptor 1 precursor (BFGF-R)

(9) Q15426 protein-tyrosine phosphatase, receptor-type, H precursor (EC 3.1.3.48)

(10) PTPM human protein-tyrosine phosphatase mu precursor (EC 3.1.3.48) (R-PTP-MU).

(11) PDGS human alpha platelet-derived growth factor receptor precursor (EC 2.7.1.112)

(12) FGR4 human fibroblast growth factor receptor 4 precursor (FGFR-4) (EC 2.7.1.112)

(13) FGR2 human fibroblast growth factor receptor 2 precursor (FGFR-2) (EC 2.7.1.112)

(14) Q13635 patched protein homolog (PTC)

(15) MANR human macrophage mannose receptor precursor.

(16) LRP2 human low-density lipoprotein receptor-related protein 2 precursor (megalin)

(17) IDD human integral membrane protein dgcr2/idd precursor (KIAA0163)

(18) AMFR human autocrine motility factor receptor precursor (AMF receptor) (gp78)

(19) ACH5 human neuronal acetylcholine receptor protein, alpha-5 chain precursor.

(20) KKIT human: stem cell growth factor receptor (proto-oncogene tyrosine-protein kinase kit) (C-KIT) (CD117 antigen)

(21) TPOR human: thrombopoietin receptor precusor (TPO-R) (myeloproliferative leukemia protein (C-MPL). TPOR or MPL.

(22) TPOR mouse: thrombopoietin receptor precursor (TPO-R) (myeloproliferative leukemia protein) (C-MPL). TPOR or MPL.

(23) Acetylcholine R

(24) Acetylcholine R alpha-5

(25) C-C chemokine receptor 6

(26) Middle T antigen

(27) integrin alpha 1

(28) FGFR2 (KGF R)

(29) FGFR1 (flg)

(30) FGFR5

(31) Erb4

(32) Vaccinia virus protein A36R

(33) Macrophage mannose R (MRC1)

(34) LDLR

(35) VLDL (rat)

(36) LRP1 low density lipoprotein receptor-related protein 1

(37) integrin beta 1

(38) integrin beta 7

(39) integrin beta 3

(40) integrin beta 5

(41) integrin beta 6

(42) G-CSFR1 (second)

(43) G-CSFR1

(44) g-csf-r

(45) IL-6B (gp130)

(46) LeptinR

(47) ProlactinR

(49) irs-1
(50) IGFl R
(51) flt3 R
(52) VEGFR2 (FLK1)
(53) PDGF R-alpha
(54) IL-9R
(55) Beta R

or a functional equivalent or analogue thereof.

The receptor is preferably a cytokine receptor. More preferably it is the GM-CSF/IL-3/IL-5 receptor which includes βc.

The binding capacity of the motif may be analysed by any binding studies or experiments available to the skilled addressee. Such experiments may include measuring the binding ability of a designated cytoplasmic protein to the binding motif. For instance electrophoretic mobility shift assays (EMSA or band shift assays) or foot print assays or pull down experiments are available to measure specific binding.

The motif (N-X-X-Y) can be identified by the presence of a tyrosine preferably in an amino acid sequence N-X-X-Y, and the ability to bind a designated cytoplasmic protein. The designated cytoplasmic protein may be Shc, SHIP-2 or any cytoplasmic protein capable of binding a further signalling molecule which activates a cascade of events leading to cell signalling pathways and biological functions such as mitogenesis, proliferation, transformation, differentiation and cell survival. More preferably, the cytoplasmic protein is Shc or SHIP-2.

Preferably, the receptor is the GM-CSF/IL-3/IL-5 receptor which includes the common beta chain (β_c). It is found that the cytokines GM-CSF, IL-3 and IL-5 signal their actions through the surface receptor via the β_c. Most preferably, the binding motif comprises a sequence which includes the amino acids Tyr

corresponding to amino acid Tyr577 of β_c according to Figure 6 or a functional equivalent or analogue thereof.

The term "functional equivalent or analogue thereof" as used herein means a sequence which functions in a similar way but may have deletions, additions or substitutions that do not substantially change the activity or function of the sequence.

The common β chain (β_c) is a component of a cytokine receptor and is part of a signalling subunit of the receptor. It is thought that the cytokine signals its functions through β_c, initiating events which cascade and culminate in an identifiable biological function such as cell survival, proliferation, differentiation and mature cell effector functions. However, the present invention is not limited to motifs of β_c but includes motifs of receptors and other proteins within the cell having similar sequences to the β_c and including a tyrosine residue at an equivalent position. It is within the scope of this invention that the bidentate motif will have the structure identified above and through this structure, the cytokine may exert its effects on the cell. Preferably, the binding motif is found in the region of the βc which includes Tyr577. Having this as a guide the binding motif of all proteins having a similar motif which corresponds to the region of βc including Tyr577 are within the scope of this invention.

The region or motif including amino acid Tyr577 of β_c or functional equivalent thereof may include residues which preferably interact with a cytoplasmic protein selected from the group including 14-3-3 protein, Shc, SHIP-2, WW-domain of the prolyl isomerase, Pin1, and the ubiquitin ligase, NEDD4 or any cytoplasmic protein capable of binding a further signalling molecule which activates a cascade of events leading to cell signalling pathways and biological functions such as mitogenesis, proliferation, transformation, differentiation and cell survival. However the present invention is not limited to this sequence but includes other equivalent sequences capable of performing the same function.

Throughout the description and claims of this specification, use of the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

The bidentate motif may be present in any type of cell. The motif structure including the amino acid sequence as described above can be screened in any cell. However, preferably it is a cell that can be affected by GM-CSF or includes βc. Most preferably, the cell is one that is affected by binding of signalling molecules to βc which harbours Tyr, more preferably corresponding to Tyr577 of βc. Most preferably, the cell is a haematopoietic cell such as, but not limited to, lymphoid, myeloid and erythroid cells. Specifically, the lymphoid lineage, comprising B cells and T cells, produces antibodies, regulates cellular immunity, and detects foreign agents such as disease-causing organisms in the blood. The myeloid lineage, which includes monocytes, granulocytes, and megakaryocytes, monitors the blood for foreign bodies, protects against neoplastic cells, scavenges foreign materials, and produces platelets. The erythroid lineage includes red blood cells, which carry oxygen. Accordingly, because the bidentate motif most preferably affects the haematopoietic cell lines, it is within the scope of the present invention that cellular activities associated with any of these cell lines may also be modulated by targeting a modification to Tyr577 of βc of GM-CSF/IL-5/IL-3.

In another embodiment of the present invention, it is preferred that the motif is selected from any one of the following sequences:

NGPYLG.....PP..HSRSLP
NVHYRT.....P...KTHTMP
**RYFTQKEE.....TESGSGP
NKKYELQDRDVCE....P.RYRSVSEP
NPTYSVM.....RSHSYP
NIFYLIR...KSGSFPMPELKLSISFP
NEEYLDLSQ.....PLEQYSPSYP
NQEYLDLSM.....PLDQYSPSFP
NATYKVD.....VIQRTRSKP
NPEY.....HSASSGP
NPDY.....WNHSLP
NPSYSSNPFVNYN....KTSICSKSNP
NTLY.....FNSQSSP

NPVYLKTTEEDLSIDIG..RH.SASVG
NPTYKMYEGGEPDDVGGLLDADFALDPDKPTNFTNPVY
NPIY.....KSAVTTVV
NPLY.....KSAITTTV
NPLY.....KEATSTFT
NPLY.....RKPISTHT
NPLY.....RGSTSTFK
PGHYL.....RCDSTQP
VQTYVLQ.....GDPRAVSTQP
QVLYGQLL.....GSPTSP
HSGYRHQVPSVQVF.....SRSESTQP
WKMYEVYDA.....KS.KSVSLP
KIPYFHA.....GGS.KCSTWP
ELDYCLKGLKL.....P.S.RTWSPP
SGDYMPM.....SPKSVSAP
SFYYSEENKLPEPEELDLEPENMESVP(LDPSASSSSLP)1283=survl.
EEIYIIM.....QSCWAFDSRKRPSFP
ISQYLQN.....S.KRKSRP
GTAY.....GLSRSQP
***YLPQEDWAP.....TSLTRP
LVAYIAFKRWNSCKQN...KQGANSRPVNQTPPPEGEKLHSDSGIS

The sequences may correspond to the following:

betaR	NGPYLG.....PP..HSRSLP
Acetylcholine R (ISOFROM?)	NVHYRT.....P...KTHTMP
Acetylcholine R alpha-5	**RYFTQKEE.....TESGSGP
C-C chemokine receptor 6	NKKYELQDRDVCE....P.RYRSVSEP
Middle T antigen	NPTYSVM.....RSHSYP
integrin alpha 1	NIFYLIR...KSGSFPMPELKLSISFP
FGFR2 (KGF R)	NEEYLDLSQ.....PLEQYSPSYP
FGFR1 (flg)	NQEYLDLSM.....PLDQYSPSFP
FGFR5	NATYKVD.....VIQRTRSKP
Erb4	NPEY.....HSASSGP
Erb4 (second)	NPDY.....WNHSLP
Vaccinia virus protein A36R	NPSYSSNPFVNYN....KTSICSKSNP
Macrophage mannose R (MRC1)	NTLY.....FNSQSSP
LDLR	NPVYQKTTEDEVHI...CHNQDGYSYP
VLDL (rat)	NPVYLKTTEEDLSIDIG..RH.SASVG
LRP1 low density lipoprotein receptor-related protein 1	NPTYKMYEGGEPDDVGGLLDADFALDPDKPTNFTNPVY
integrin beta 1	NPIY.....KSAVTTVV (end of protein)
interin beta 7	NPLY.....KSAITTTV (end of protein)
integrin beta 3	NPLY.....KEATSTFT (end of protein)
integrin beta 5	NPLY.....RKPISTHT (end of protein)
integrin beta 6	NPLY.....RGSTSTFK
G-CSFR1 (second)	PGHYL.....RCDSTQP
G-CSFR1	VQTYVLQ.....GDPRAVSTQP
g-csf-r	QVLYGQLL.....GSPTSP (CHECK?)
IL-6B (gp130)	HSGYRHQVPSVQVF.....SRSESTQP
leptinR.	WKMYEVYDA.....KS.KSVSLP
prolactinR...	KIPYFHA.....GGS.KCSTWP
insulinR	ELDYCLKGLKL.....P.S.RTWSPP
irs-1	SGDYMPM.....SPKSVSAP

flt3 R	EEIYIIM.....QSCWAFDSRKRPSFP
VEGFR2 (FLK1)	ISQYLQN.....S.KRKSRP
PDGF R-alpha	GTAY.....GLSRSQP
IL-9R	***YLPQEDWAP.....TSLTRP
p75 NTR	LVAYIAFKRWNSCKQN...KQGANSRPVNQTPPPEGEKLHSDSGIS

More particularly, the binding motif or an equivalent may have any one of the following sequences.

NGPY
NVHY
**RY
NKKY
NPTY
NIFY
NEEY
NQEY
NATY
NPEY
NPDY
NPSY
NTLY
NPVY
NPIY
NPLY
■

The present invention has found that mutation of Tyr577 is required to abolish haematopoetic cell survival in response to GM-CSF. This would suggest that this residue is able to independently regulate cell survival.

Applicants have further found that Shc interacts with 14-3-3 via a Tyr 179 which is necessary for PI-3 kinase activity. Via this signalling molecule, further signalling pathways are activated leading to cellular activities such as mitogenesis, proliferation, transformation, differentiation, cell survival, chemotaxis, motility, enhanced phagocytosis, bacterial killing, superoxide production and cytotoxicity.

In another aspect of the present invention there is provided a method of modulating activity in a cell, said method including:

introducing a modification to a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

wherein X is any residue, and Y is tyrosine.

In a preferred aspect of the present invention, there is provided a method of modulating activity in a cell, said method including

introducing a modification to a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine and wherein the tyrosine is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor or an equivalent thereof.

The use of the term "equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor" or "Tyr 577 or an equivalent" means Tyr577 of βc or another residue, preferably tyrosine which is in a position that can be aligned with Tyr577 based on the amino acid sequence of the common βc as in Figure 6. The equivalent is to behave in a similar manner and have the same effects as Tyr577 on the common βc.

A single amino acid corresponding to Tyr577 of βc of GM-CSF receptor has been identified as a controlling factor in the regulation of cellular activities. For this reason, this invention targets this amino acid residue and the motif surrounding and supporting this residue for modulating cellular activity.

The GM-CSF receptor is responsible for a number of cellular activities, most of which cascade from binding of cytoplasmic proteins to βc of the receptor. Engagement of the signalling molecules to βc results in the stimulation of a number of cellular activities.

The common β chain (β_c) is a component of a cytokine receptor and is a signalling subunit of the receptor. It is thought that the cytokine signals its functions through the β_c, initiating events which cascade and culminate in an identifiable biological function such as cell survival, proliferation, differentiation and mature cell effector functions. However, the present invention is not limited

to β_c of GM-CSF but includes motifs of receptors having similar sequences to β_c and including a residue in a similar position to the Tyr577 residue.

"Cellular activity" or "activity in the cell" as used herein may be selected from the group including cell survival, proliferation, differentiation, mitogenesis, transformation, chemotaxis, motility, enhanced phagocytosis, enhanced bacterial killing, superoxide production and cytotoxicity. Preferably, the cellular activity is any activity directly related to the GM-CSF receptor. Most preferably, the cellular activity is cell survival and proliferation or activity that leads to cell growth and colony growth.

However, these activities can be modulated by targeting a Tyr577 or an equivalent residue of β_c as a controlling factor of the cellular activities. This residue has previously been implicated in coupling the receptor to the adaptor protein Shc and to the tyrosine phosphatase SHIP-2.

"Modulation" or "modulating" as used herein with respect to cellular activity means modifying, altering or changing the activity compared to unmodified levels. The activity may be increased or decreased. For instance, proliferation may be increased or decreased. The modulation may cause an enhancement or reduction of the cellular activity. In any case, the cellular activity is changed by virtue of modification of the receptor at amino acid residue Tyr577 of β_c.

Any cell may be affected by a modification to a tyrosine, preferably Tyr577 of the common beta chain (βc). However, it is generally a cell that can be affected by GM-CSF or includes βc. Most preferably, the cell is one that is affected by binding of signalling molecules to βc which harbours Tyr577. Most preferably, the cell is a haematopoietic cell such as, but not limited to, lymphoid, myeloid and erythroid cells. Specifically, the lymphoid lineage, comprising B cells and T cells, produces antibodies, regulates cellular immunity, and detects foreign agents such as disease-causing organisms in the blood. The myeloid lineage, which includes monocytes, granulocytes, and megakaryocytes, monitors the blood for foreign bodies, protects against neoplastic cells, scavenges foreign

cells, which carry oxygen. Because the Tyr577 most preferably affects the haematopoietic cell lines, it is within the scope of the present invention that cellular activities associated with any of these cell lines may also be modulated by targeting a modification to Tyr577 of βc of GM-CSF.

The cell as used herein may be an isolated cell or be a cell contained within tissue or bodily fluids. Preferably the cell is a haematopoetic cell as herein described. Generally the cell will have a receptor for a cytokine such as GM-CSF. Most preferably the cell will have the GM-CSF receptor and activate via βc.

The haematopoietic cells may be derived from any source, such as from the bone marrow or peripheral blood, cell cultures or tissue samples. They may be isolated by methods available to the skilled addressee.

The methods of the present invention require modulation of at least one cellular activity by introducing a modification to a Tyr, preferably an equivalent to Tyr577 of βc. Introduction of the modification as used in the present invention may include introducing a mutation to a residue equivalent to Tyr577 of βc or it may include transducing a βc mutation having a mutation at the position of Tyr577 on βc. Methods of blocking the effects of Tyr577 to modulate cellular activity are also within the scope of the present invention and in the spirit of introducing a modification to Tyr577. In this case it includes molecules that are directed to the Tyr577 residue to block binding of Shc or SHIP-2 to activate the cascade of events arising from the binding or it may include preventing phosphorylation of Tyr577.

Preferably Tyr577 is the sole target and modulation of the cellular activity is dependent on the modification of Tyr577 only.

In one preferred embodiment, the modification is by inducing a mutation at a position equivalent to Tyr577 on βc. The mutation may include a substitution, deletion, or insertion of another amino acid such that the position equivalent to

Tyr577 on βc is debilitated and no longer functional in so far as it cannot perform its normal functions such as binding cytoplasmic proteins.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, i.e., conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

"Deletions" result from the amino acid being physically removed. The position may be targeted by methods available to the skilled addressee as used in site directed mutagenesis.

"Insertions" may arise where a similar amino acid is not inserted but another amino acid is inserted. Hence it is a non-conservative amino acid change.

Preferably, the substitutions replace Tyr577 or an equivalent with another amino acid. Preferably the Tyr577 or an equivalent is replaced with phenylalanine (Tyr577Phe).

In yet another preferred embodiment, the modification of Tyr577 or an equivalent may be achieved using antagonists, inhibitors, mimetics or derivatives of the Tyr577 or an equivalent. The terms "antagonist" or "inhibitor", as used herein, refer to a molecule which, when bound to Tyr577 or an equivalent, blocks or modulates the activity of Tyr577 or an equivalent. Antagonists and inhibitors may include proteins, nucleïc acids, carbohydrates, antibodies or any other molecules including ligands which bind to Tyr577 or an

equivalent. Other modulators of the activity and/or expression of Tyr577 or an equivalent include a range of rationally-designed, synthetic inhibitors.

Modifications at Tyr577 or an equivalent may be achieved by direct or indirect methods. Modulation of activity of Tyr577 or an equivalent may be achieved using direct methods known to those of skill in the art and include, but are not limited to, knockout technology, targeted mutation, gene therapy. Indirect methods for modulating activity of Tyr577 or an equivalent may include targeting upstream or downstream regulators such as regulators of the cytoplasmic protein Shc or SHIP-2.

In yet another preferred embodiment, the modification of the Tyr577 or an equivalent that modulates cellular activity is achieved by introducing a construct to the cell which contains a βc mutant having a mutation at the Tyr577 or an equivalent position.

Applicants have found that a single amino acid substitution in the common beta chain of the GM-CSF/IL-3/IL-5 receptors has a profound effect on haematopoietic cell behaviour that results in increased cell survival and proliferation. Primary foetal liver cells from mice devoid of endogenous beta common and beta IL-3 were used and transduced with an IRES construct containing both the alpha chain and the beta chain of the human GM-CSF receptor. Foetal liver cells transduced with a beta chain mutant Tyr577Phe exhibited increased survival both in the absence and in the presence of human GM-CSF. In addition they formed a much larger number of day 14 colonies which were larger in size. Delta assays showed an expansion of day 14 colony-forming cells after seven days in liquid culture with GM-CSF. Increased cell proliferation was also seen in CTL-EN cells transduced with the Tyr577Phe mutant receptor as demonstrated by a shift to the left in the response to human GM-CSF. Tyr577Phe mutant did not alter the recruitment of SHIP-2 to the receptor, the activation of JAK-2 or the phosphorylation of STAT-5, however, mutation of Tyr577 failed to recruit Shc to the receptor and to promote its tyrosine phosphorylation.

Significantly, a profound effect on the activation of raf and in the activation of the SH-2 -containing inositol phosphatase SHIP was found.

Cells having an introduced mutation of the Tyr577 or an equivalent residue of the receptor, and preferably the βc of the receptor may be used in assays to screen for compounds that can affect cell survival.

In a further preferred embodiment, the present invention provides a method of modulating proliferation in a cell said method including

introducing a modification to a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

In a further preferred embodiment of the present invention, there is provided a method of modulating proliferation in a cell, said method including

introducing a modification to a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine and wherein the tyrosine is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor or an equivalent thereof.

Modification of the Tyr577 or an equivalent may be conducted by any means herein described. However, any means that uncouples an interaction of Tyr577 or an equivalent to the cytoplasmic protein Shc or SHIP-2 will be most preferred to cause modulation of proliferation. Full uncoupling of the Shc or SHIP-2 cytoplasmic protein may lead to complete inhibition of phosphorylation of the Tyr577 or an equivalent. The applicants have found that debilitating this event leads to an increased cellular activity and proliferation resulting in increased survival and larger colonies.

Accordingly, in yet another preferred embodiment, the invention provides a

modifying phosphorylation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

In a further preferred embodiment of the present invention, there is provided a method of modulating activity in a cell, said method including

modifying phosphorylation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine and wherein the tyrosine is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor or an equivalent thereof.

Preferably the cellular activity is cell growth and/or proliferation. Both activities can lead to larger cells and larger colonies.

The modulation of the phosphorylation events which phosphorylate the tyrosine on the binding motif will affect the binding of a cytoplasmic protein which in turn will affect the activation of signalling molecules which activate a cascade of events leading to cell signalling pathways and cellular activities. Preferably the cellular activities are selected from the group including mitogenesis, proliferation, transformation, differentiation, cell survival, chemotaxis, motility, enhanced phagocytosis, bacterial killing, superoxide production and cytotoxicity. Most preferably, the cellular activity is proliferation or cell survival. More preferably it is cell survival.

The modification of phosphorylation of the Tyr577 or an equivalent may be an increase or a decrease of the phosphorylation of the residue. Methods of increasing or decreasing (inhibiting) phosphorylation are known to those skilled in the art. However, specifically, the use of specific kinase inhibitors are

Most preferably Tyr577 of βc is modified by phosphorylation. By inhibiting phosphorylation of Tyr577 cellular proliferation can be increased. Conversely, by inducing phosphorylation, cell growth can be inhibited.

Although not wishing to be limited by theory, it is perceived that phosphorylation of Tyr577 of βc may improve the binding of a cytoplasmic protein such as Shc or SHIP-2 to the residue so that when the cytoplasmic protein is reacted with the residue or equivalent thereof, binding may occur to bring other cytoplasmic proteins or signalling molecules into close proximity to the receptor. Phosphorylation may occur by any means which transfers a phosphoryl (phosphate) group to the Tyr577.

In yet another preferred aspect of the present invention, there is provided a method of increasing cell growth, said method including

inhibiting activation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

In a further preferred embodiment of the present invention, there is provided a method of increasing cell growth, said method including

inhibiting activation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine and wherein the tyrosine is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor or an equivalent thereof.

As found in the present invention the tyrosine of the motif and more preferably Tyr577 or an equivalent is pivotal in the activation of mechanisms that are essential to the survival of cells and most preferably of cell growth and proliferation. Increased cell growth leads to increased cell colonies and the

The cells are most preferably haematopoietic cells as described above, and are cells that incorporate the GM-CSF receptor and more specifically, the common βc. However, other cells may also be modified using the motif as herein described.

It has been described above that loss of effect of Tyr577 or an equivalent either by substitution, deletion or insertion, inhibition or by non-activation of phosphorylation, can cause an increase in cell growth and colony size. These forms of inhibiting activation of Tyr577 or an equivalent are within the scope of the present invention.

It is preferred that antagonists that bind to Tyr577 or an equivalent in either the phosphorylated or unphosphorylated form can be used to inhibit activation of Tyr577 or an equivalent. By this it is meant that Tyr or Tyr577 or an equivalent can lose its ability to bind a cytoplasmic protein and further activate cell signalling pathways. This may be useful to increase cell survival or activation. Preferably antagonists may be useful to increase cell survival or activation by preventing phosphorylation preferably by preventing Tyr577 or an equivalent phosphorylation of β_c or equivalent thereby preventing the cytoplasmic protein binding to the binding motif. Alternatively, they may prevent the interaction of a signalling molecule binding to a phosphotyrosine bound Shc or equivalent.

Prevention of phosphorylation of β_c or Tyr577 or an equivalent as described above may also inhibit activation of Tyr577 or an equivalent and this may be achieved by inhibition of the specific kinases involved in the phosphorylation of the Tyr577 or an equivalent residue or it may include mutation of the residue to prevent the cytoplasmic protein such as Shc or SHIP-2 from binding and activating cell cycle pathways. Kinase inhibitors such as H89 which binds to PKA may be used. Preferably, cell permeable kinase inhibitors are used.

Antagonists may include antibodies, small peptides, small molecules, peptide mimetics or any type of molecule known to those skilled in the art that are

cytoplasmic proteins such as Shc, SHIP-2 to a Tyr residue or a signalling molecule. Antibodies may be generated in response to any of the Tyr577 or an equivalent described above by methods known and available to the skilled addressee.

However, the invention may further encompass modifying the cytoplasmic proteins that also bind to Tyr577 or an equivalent. Without affecting Tyr577 or an equivalent in the cell, modifications can be directed at the cytoplasmic proteins or other factors which may cause phosphorylation of the Tyr577 or an equivalent. Effectively, the invention includes any means which can cause an uncoupling of the interaction between Tyr577 or an equivalent and its cytoplasmic proteins, namely Shc and SHIP-2.

In yet another aspect of the present invention there is provided a method of transplantation of cells, said method including

inhibiting activation in the cell of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine; and

transplanting the cells into a patient in need of such treatment.

In a further preferred embodiment of the present invention, there is provided a method of transplantation of cells, said method including

inhibiting activation in the cell of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine and wherein the tyrosine is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor or an equivalent thereof; and

transplanting the cells into a patient in need of such treatment.

This method of inhibiting activation of Tyr577 or an equivalent to increase cell growth may be conducted *in vivo* or *in vitro*. Cell culture techniques to increase cell populations may be enhanced by the method described herein, by inhibiting the interaction of Tyr577 or an equivalent and the cytoplasmic protein Shc or SHIP-2. This would be particularly useful for expanding colonies of cells and progenitor cells *in vitro* prior to injection into a patient. However the interaction of Tyr577 or an equivalent may be directed *in vivo* by the use of specific kinase inhibitors.

The inhibition of the activation in the cell may be useful for enhancing transplantation efficiency. By increasing proliferation of the cell by inhibiting activation, more cells may successfully incorporate and integrate in the transplant.

Similarly, cells in the region of the transplantation may be treated to inhibit activation Tyr577 or an equivalent so as to increase cell proliferation and growth in that region to enhance cell growth and reduce rejection of the transplant. This may assist in the graft "taking" and possibly reduce the number of cells required to transplant. If a lesser number of cells is transplanted, the method is useful to expand colony forming cells which is useful for providing protection after bone marrow transplantation, particularly in the time between stem cell engraftment and bone marrow recovery.

Patients undergoing transplantation may be treated with inhibitors of phosphorylation and directed toward the area of transplantation so as to increase cell growth or enhance cell growth which may improve the chances of the grafted tissue for survival.

In another aspect of the present invention there is provided a method of improving wound healing in a patient, said method including

inhibiting activation of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any amino acid and Y is tyrosine in one or more of the

In a further preferred embodiment of the present invention, there is provided a method of transplantation of cells, said method including

inhibiting activation in the cell of a tyrosine of a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine and wherein the tyrosine is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF receptor or an equivalent thereof in a region of the wound.

By inhibiting activation of Tyr577 or an equivalent of βc, the cells can be induced to proliferate in the region of the wound. As described above, there are methods for inhibiting the activation of Tyr577 or an equivalent and any of these methods described above may be used to inhibit Tyr577 or an equivalent and therefore uncouple Tyr577 from Shc or SHIP-2. Most preferred is an inhibition of phosphorylation of Tyr577 or an equivalent. Preferably, this may be achieved by using specific kinase inhibitors.

However, since phosphorylation is a process that is common to a number of cellular activities, the use of a general dephosphorylating agent may be harmful.

Accordingly, it is also preferred to utilise antagonists of Tyr577 or an equivalent which may inhibit the binding of Tyr577 or an equivalent to the cytoplasmic protein Shc or SHIP-2. The use of these molecules can target and specifically direct their inhibition of the phosphorylation and subsequent events which can assist in the increased cell growth and proliferation for wound healing.

In yet another aspect of the present invention, there is provided a method for screening cell growth promoting compounds, said method including

providing a cell having a receptor containing a βc having a Tyr577 residue or equivalent;

inducing phosphorylation of the Tyr577 or an equivalent;

assessing colony formation of the cells.

This aspect of the present invention utilises the finding that Tyr577 or an equivalent is central to the induction of cell growth and survival leading to larger colonies and that inhibition of phosphorylation is necessary to increase cell survival and produce larger colonies. Where the compound increases colony formation and reverses the effects of the induced phosphorylation, then it may be deduced that the compound has growth promotion properties.

Preferably the cell is a haematopoietic cell as herein described.

The present invention may also be used as a model for proliferative diseases. Given the interaction between the binding motif and the cytoplasmic proteins, any part of the interactions can be monitored to determine any aberration between the cells in question and that of a normal cell. Aspects of the model may analyse the phosphorylation ability of the Tyrosine residues or analyse the interaction between the respective cytoplamic proteins of Shc and SHIP-2 both with Tyrosine.

Preferably the model is based on a haematopoetic cell as described above. However, any other cell that contains the motif may be used. More preferably, the cell has a GM-CSF receptor including the Tyr577 or an equivalent upon which the model is based.

The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

Examples of the procedures used in the present invention will now be more fully described. It should be understood, however, that the following description is

illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

EXAMPLES

Example 1: Y577F mutation leads to enhanced numbers of colonies in response to GM-CSF

(a) Cell lines and primary cells

The murine factor dependent cell line CTL which has been described previously {Le, Stomski, et al. 2000 4135 /id} was used for the proliferation studies. These cells were maintained in RPMI-1640 with 10% foetal calf serum (FCS) and murine IL-2 generated from an *E-coli* expression system.

Psi-2 ecotropic retrovirus packaging cells {Mann, Mulligan, et al. 1983 3888 /id} were maintained in DMEM supplemented with 10% FCS. Transfected pools of cells were selected and maintained in the above medium plus geneticin sulphate (G418) at 400 μg/ml. were transfected with the RufNeo retroviral vector {Rayner & Gonda 1994 4136 /id} containing wild type or mutant hβc.

Murine foetal liver cells were harvested at dpc 13.5 from mice that were knockout for both the common beta chain (β_c) and the IL-3-specific beta chain (β_{IL-3}) {Scott, Robb, et al. 2000 2960 /id}. These cells were cultured in IMDM supplemented with 15% FCS.

(b) Generation of murine cells expressing human GM-CSF receptor

CTL-EN or murine foetal liver cells were engineered to express both alpha and beta chains of the GM-CSF receptor using a retroviral transduction system.

For generation of the retroviral particles, the backbone plasmid was based on pRUFneo and the GM-CSF receptor subunits were cloned into the multiple cloning site. Additionally an IRES was inserted to allow expression of both receptor subunits in a single infection event. Producer cell lines were generated in psi-2 cells by calcium phosphate transfection followed by FACS to generate cell lines with high expression of both receptor subunits.

Target cells (CTL or foetal livers) were co-cultivated with the irradiated psi-2 cells for 48 hours and then harvested and cultured for an additional 24 hours with fresh medium added. Transduction levels were assessed using immunofluorescence analysis with biotinylated antibodies specific to the GM-CSF receptor alpha chain and streptavidin-PE. 5000-10000 cells were analysed using a Coulter Epics XL.

(c) Colony Assays

The response of immature cells to GM-CSF was analysed using colony formation assays. Foetal liver cells were transduced and expression of the GM-CSF receptor quantitated as described above. Colony forming cells were assayed using a double layer agar assay. Plates were prepared with underlayers comprised of IMDM supplemented 0.5% agar (Difco) containing cytokines as shown in the figures. An overlayer was added with transduced cells, at a concentration of 100,000 per dish, in 0.3% agar. All media contained 10% heat inactivated FCS (JRH Biosciences) with penicillin and streptomycin added.

All cytokines were diluted in PBS and a control of PBS alone was added in each assay to verify that colonies were formed in response to cytokine stimulation. Additionally, all assays included a general stimulus composed of a cocktail of IL-6 (100 ng/ml), SCF (100 ng/ml) and erythropoietin (4U/ml).

Plates were incubated at 37°C 5% CO_2 for 14 days and colonies counted using an inverted microscope at the completion of this time.

Figure 1 shows colony formation from foetal livers transduced with GM-CSF receptors. 100,000 cells were plated in 35mm dishes with GM-CSF at 100 ng/ml. Colony numbers were enumerated after incubation at 37°C 5% CO_2 for 14 days. The figure shows data from 9 separate experiments with triplicate plates in each. Average and standard deviations are represented in the figure.

Example 2: Y577F point mutation can give rise to larger colonies than wild type GM-CSF receptor beta chain.

Figure 2 shows colonies from 2 of the assays shown in Example 1 were analysed according to their overall size. Stained dishes were categorised into 3 groups; 1), colonies greater than 2 mm 2) between 1 and 2 mm and 3) those smaller than 1 mm. Data is represented for each group as the percent of the total number of colonies and figure 2 shows mean value with standard deviation.

Example 3: Foetal liver cells transduced with Y577F mutant of the beta chain form greater numbers of colonies at all concentrations of GM-CSF.

A titration of GM-CSF was used to assess the response of the cells to lower concentrations of cytokine in addition to the higher concentrations reported above. Figure 3 shows that there are greater numbers of colonies.

Example 4: Delta Assay of cells cultured for 7 or 14 days prior to colony formation

(a) Delta Assay

An extended colony assay was performed in which cells were either plated as described above on Day 0 or cultured in GM-CSF or a cocktail of IL-6 (100 ng/ml), SCF (100 ng/ml) and G-CSF (10 ng/ml) for 7 or 14 days. On Days 7 or 14 cells were plated in colony assays exactly as previously. All colonies were enumerated after 14 days incubation at 37°C 5% CO_2.

(b) 7 days prior to colony formation

Cells were cultured in liquid media supplemented with either GM-CSF or cocktail for 7 days prior to plating in agar. As can be seen from the groups grown in GM-CSF and in Figure 4a, as denoted by GM on the x-axis, there is greater response to cocktail stimulation for the Y577F group compared to the wild type receptor. Response is similar for cells grown in cocktail for the 7 days prior to plating.

(c) 14 days prior to colony formation

Cells were cultured in liquid media supplemented with either GM-CSF or cocktail for 14 days prior to plating in agar. As can be seen from the groups grown in GM-CSF and in Figure 4b, as denoted by GM on the x-axis, there is greater response to cocktail stimulation for the Y577F group compared to the wild type receptor. Response to cocktail but not GM-CSF is also greater for this group for cells grown in cocktail for the 14 days prior to plating.

Example 5: CTL cells expressing the human GM-CSF receptor have greater response to GM-CSF when Y577 is mutated to phenylalanine.

(a) Proliferation Assay

The proliferative response of CTL cells expressing wild type or mutant GM-CSF receptor beta chains was measured in microwell assays of 1000 cells stimulated with serially diluted quantities of GM-CSF. CTL cells are grown in IL-2 but were starved of growth factor for 24 hours before setting up the proliferation assays as described previously {Sun, Woodcock, et al. 1996 3188 /id}. The ^{3}H-Thymidine incorporation was determined by liquid scintillation.

Figure 5 shows a proliferation assay for CTL cells transduced with the human GM-CSF receptor. The cells were starved of cytokine for 24 hours and then stimulated with a titration of human GM-CSF or murine IL-2 for 48 hours. Proliferation was quantitated using ^{3}H-Thymidine incorporation and liquid scintillation counting. The reduction in ED50 for can clearly be seen for Y577F when compared to wild type beta chain for response to GM-CSF but not for IL-2.

Finally it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

CLAIMS

1. A binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine or an equivalent thereof.

2. A binding motif of a receptor molecule capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine or an equivalent thereof.

3. A binding motif according to claim 1 or 2 derived from a receptor selected from the group including:

(1) GM-CSF/IL-3/IL-5 receptor

(2) IL6 human interleukin-6 receptor beta chain precursor (IL-6R-beta)

(3) LEPR human leptin receptor precursor (LEP-R) (OB RECEPTOR) (OB-R).

(4) TNR2 human tumor necrosis factor receptor 2 precursor (tumor necrosis factor

(5) VGR1 human vascular endothelial growth factor receptor 1 precursor

(6) TRK3 human receptor protein-tyrosine kinase TKT precursor (EC 2.7.1.112)

(7) Q01974 protein-tyrosine kinase transmembrane receptor ROR2 precursor

(8) FGR1 human basic fibroblast growth factor receptor 1 precursor (BFGF-R)

(9) Q15426 protein-tyrosine phosphatase, receptor-type, H precursor (EC 3.1.3.48)

(10) PTPM human protein-tyrosine phosphatase mu precursor (EC 3.1.3.48) (R-PTP-MU).

(11) PDGS human alpha platelet-derived growth factor receptor precursor (EC 2.7.1.112)

(12) FGR4 human fibroblast growth factor receptor 4 precursor (FGFR-4) (EC 2.7.1.112)

(13) FGR2 human fibroblast growth factor receptor 2 precursor (FGFR-2) (EC 2.7.1.112)

(14) Q13635 patched protein homolog (PTC)

(15) MANR human macrophage mannose receptor precursor.

(16) LRP2 human low-density lipoprotein receptor-related protein 2 precursor (megalin)

(17) IDD human integral membrane protein dgcr2/idd precursor (KIAA0163)

(18) AMFR human autocrine motility factor receptor precursor (AMF receptor) (gp78)

(19) ACH5 human neuronal acetylcholine receptor protein, alpha-5 chain precursor.

(20) KKIT human: stem cell growth factor receptor (proto-oncogene tyrosine-protein kinase kit) (C-KIT) (CD117 antigen)

(21) TPOR human: thrombopoietin receptor precusor (TPO-R) (myeloproliferative leukemia protein (C-MPL). TPOR or MPL.

(22) TPOR mouse: thrombopoietin receptor precursor (TPO-R) (myeloproliferative leukemia protein) (C-MPL). TPOR or MPL.

(23) Acetylcholine R

(24) Acetylcholine R alpha-5

(25) C-C chemokine receptor 6

(26) Middle T antigen

(27) integrin alpha 1

(28) FGFR2 (KGF R)

(29) FGFR1 (flg)

(30) FGFR5

(31) Erb4

(32) Vaccinia virus protein A36R

(33) Macrophage mannose R (MRC1)

(34) LDLR

(35) VLDL (rat)

(36) LRP1 low density lipoprotein receptor-related protein 1

(37) integrin beta 1

(38) integrin beta 7

(39) integrin beta 3

(40) integrin beta 5

(41) integrin beta 6

(42) G-CSFR1 (second)

(43) G-CSFR1

(44) g-csf-r

(45) IL-6B (gp130)

(46) LeptinR

(47) ProlactinR

(48) insulinR

(49) irs-1

(50) IGFI R

(51) flt3 R

(52) VEGFR2 (FLK1)

(53) PDGF R-alpha

(54) IL-9R

(55) BetaR

or a functional equivalent or analogue thereof.

4. A binding motif or equivalent thereof according to any one of claims 1 to 3 derived from a sequence selected from the group including:

NGPYLG.....PP..HSRSLP
NVHYRT.....P...KTHTMP
**RYFTQKEE.....TESGSGP
NKKYELQDRDVCE....P.RYRSVSEP
NPTYSVM.....RSHSYP
NIFYLIR...KSGSFPMPELKLSISFP
NEEYLDLSQ.....PLEQYSPSYP
NQEYLDLSM.....PLDQYSPSFP
NATYKVD.....VIQRTRSKP
NPEY.....HSASSGP
NPDY.....WNHSLP
NPSYSSNPFVNYN....KTSICSKSNP
NTLY.....FNSQSSP
NPVYQKTTEDEVHI...CHNQDGYSYP

NPVYLKTTEEDLSIDIG..RH.SASVG
NPTYKMYEGGEPDDVGGLLDADFALDPDKPTNFTNPVY
NPIY.....KSAVTTVV
NPLY.....KSAITTTV
NPLY.....KEATSTFT
NPLY.....RKPISTHT
NPLY.....RGSTSTFK
PGHYL.....RCDSTQP
VQTYVLQ.....GDPRAVSTQP
QVLYGQLL.....GSPTSP
HSGYRHQVPSVQVF.....SRSESTQP
WKMYEVYDA.....KS.KSVSLP
KIPYFHA.....GGS.KCSTWP
ELDYCLKGLKL.....P.S.RTWSPP
SGDYMPM.....SPKSVSAP
SFYYSEENKLPEPEELDLEPENMESVP(LDPSASSSSLP)
EEIYIIM.....QSCWAFDSRKRPSFP
ISQYLQN.....S.KRKSRP
GTAY.....GLSRSQP
***YLPQEDWAP.....TSLTRP
LVAYIAFKRWNSCKQN...KQGANSRPVNQTPPPEGEKLHSDSGIS

5. A binding motif according to any one of claims 1 to 4 having a sequence selected from the group including:

NGPY
NVHY
**RY
NKKY
NPTY
NIFY
NEEY
NQEY
NATY
NPEY
NPDY
NPSY
NTLY
NPVY
NPIY
NPLY

6. A binding motif according to any one of claims 1 to 5 wherein the sequence is derived from a cytokine receptor.

7. A binding motif according to any one of claims 1 to 6 wherein the receptor is the GM-CSF/IL-3/IL-5 receptor.

8. A binding motif according to any one of claims 1 to 7 wherein the sequence includes the common beta chain (βc).

9. A binding motif according to any one of claims 1 to 8 wherein the Tyr residue is equivalent to Tyr577 of the common beta chain (βc).

10. A binding motif according to any one of claims 1 to 9 having a modification at a residue equivalent to the Tyr residue.

11. A binding motif according to any one of claims 1 to 10 wherein the residue equivalent to the Tyr residue is substituted with a Phe residue.

12. A binding motif according to any one of claims 1 to 11 which binds to a cytoplasmic protein selected from the group including 14-3-3 protein, Shc, SHIP-2, WW-domain of the prolyl isomerase, Pin1 and the ubiquitin ligase, NEDD4.

13. A binding motif according to any one of claims 1 to 12 wherein the cytoplasmic protein is Shc or SHIP-2.

14. A method of modulating activity in a cell, said method including:

introducing a modification to a binding motif capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

15. A method of modulating activity in a cell, said method including:

introducing a modification to a binding motif according to any one of claims 1 to 13.

16. A method according to claim 15 wherein the tyrosine residue is equivalent to Tyr577 of the common beta chain (βc).

17. A method according to claim 16 wherein the common beta chain (βc) is of the GM-CSF/IL-3/IL-5 receptor.

18. A method according to any one of claims 15 to 17 wherein the activity is modulated by introducing a modification of phosphorylation of the Tyr of the motif.

19. A method according to claim 18 wherein the phosphorylation is increased by subjecting the cell to a phosphorylating agent.

20. A method according to claim 19 wherein the phosphorylating agent is a kinase.

21. A method according to claim 18 wherein the phosphorylation is decreased by mutating, substituting or deleting the Tyr.

22. A method according to claim 23 wherein the Tyr is substituted for Phe.

23. A method according to claim 18 wherein the phosphorylation is decreased by subjecting the cell to an antagonist which inhibits phosphorylation of the Tyr.

24. A method according to claim 18 wherein the phosphorylation is decreased by subjecting the cell to a kinase inhibitor to inhibit phosphorylation of the Tyr.

25. A method according to any one of claims 21 to 24 for inhibiting cellular activity, said method comprising decreasing or inhibiting phosphorylation of the Tyr motif.

26. A method according to claim 25 for inhibiting activity in a cell, said method comprising inhibiting binding of a cytoplasmic protein to the motif.

27. A method according to claim 26 wherein the cytoplasmic protein is selected from the group including 14-3-3 protein, Shc, SHIP-2, WW-domain of the prolyl isomerase, Pin1 and the ubiquitin ligase, NEDD4.

28. A method according to claim 27 wherein the cytoplasmic protein is Shc.

29. A method according to claim 19 or 20 for activating cellular activity, said method comprising inducing phosphorylation of the Tyr of the motif.

30. A method according to any one of claims 14 to 29 wherein the cellular activity is selected from the group including cell survival, proliferation, differentiation, mitogenesis, transformation, chemotaxis, motility, enhanced phagosytosis, enhanced bacterial killing, superoxide production and cytoxicity.

31. A method according to any one of claims 14 to 30 wherein the cellular activity is cell survival.

32. A method according to any one of claims 14 to 30 wherein the cellular activity is proliferation.

33. A method according to any one of claims 14 to 32 wherein the cell is a haematopoietic cell.

34. A method according to claim 32 for increasing proliferation, said method including inhibiting phosphorylation of the Tyr.

35. A method according to claim 32 for inhibiting proliferation, said method including inducing phosphorylation of the Tyr.

36. A method according to claim 32 for increasing cell growth, said method including inhibiting activation of the Tyr.

37. A method of modulating activity in a cell, said method including:

introducing a modification to a binding motif of a receptor capable of binding to a cytoplasmic protein said motif consisting of the following amino acid sequence:

N-X-X-Y

wherein X is any residue, and Y is tyrosine.

38. A method according to claim 34 wherein the tyrosine residue is equivalent to Tyr577 of the common beta chain (βc).

39. A method according to claim 35 wherein the common beta chain (βc) is of the GM-CSF/IL-3/IL-5 receptor.

40. A method according to claim 37 for increasing proliferation, said method including inhibiting phosphorylation of the Tyr.

41. A method according to claim 37 for inhibiting proliferation, said method including inducing phosphorylation of the Tyr.

42. A method according to claim 37 for increasing cell growth, said method including inhibiting activation of the Tyr.

43. A method for transplantation of cells, said method including

inhibiting activation of a Tyr of a binding motif according to any one of claims 1 to 13 in the cells; and

transplanting the cells.

44. A method for enhancing transplantation efficiency, said method including

inhibiting activation of a Tyr of a binding motif according to any one of claims 1 to 13 in the cells; and

transplanting the cells.

45. A method according to claim 43 or 44 wherein activation of the Tyr is inhibited *in vitro* prior to transplanting.

46. A method according to claim 43 or 44 wherein the activation of the Tyr is inhibited in a region of transplantation.

47. A method according to any one of claims 43 to 46 wherein activation is inhibited by inhibiting phosphorylation of the Tyr.

48. A method according to any one of claims 43 to 47 wherein the Tyr is equivalent toTyr577 of the common beta chain (βc) of the CM-CSF/IL-3/IL-5 receptor.

49. A method according to claim 48 wherein the common beta chain (βc) is of the GM-CSF/IL-3/IL-5 receptor.

50. A method of improving wound healing in a patient, said method including inhibiting activation of a Tyr of a binding motif according to any one of claims 1 to 13 in a region of the wound.

51. A method according to claim 36 wherein the activation is inhibited by inhibiting phosphorylation of the Tyr.

52. A method according to claim 50 or 51 wherein the Tyr is equivalent to Tyr577 of the common beta chain (βc) of the GM-CSF/IL-3/IL-5 receptor.

53. A method according to claim 52 wherein the common beta chain (βc) is of the GM-CSF/IL-3/IL-5 receptor.

54. A use of an inhibitor of activation of a Tyr of a binding motif according to any one of claims 1 to 13 in the preparation of a medicament for the treatment of a wound.

55. A use according to claim 54 wherein the inhibitor inhibits phosphorylation of the Tyr.

56. A use according to claim 54 or 55 wherein the Tyr is equivalent to Tyr577 of the common beta chain (βc).

57. A use according to claim 56 wherein the common beta chain (βc) is of the GM-CSF/IL-3/IL-5 receptor.

58. A method for screening of cell growth promoting compounds, said method including

obtaining a cell having a receptor containing a βc having a Tyr577 residue or equivalent;

inducing phosphorylation of the Tyr or an equivalent in a binding motif according to any one of claims 1 to 13;

exposing the cell to the compound; and

assessing colony formation of the cells.

59. A method according to claim 58 wherein the Tyr is equivalent to Tyr577 of the common beta chain (βc).

60. A use according to claim 56 wherein the common beta chain (βc) is of the GM-CSF/IL-3/IL-5 receptor.

Figure 1

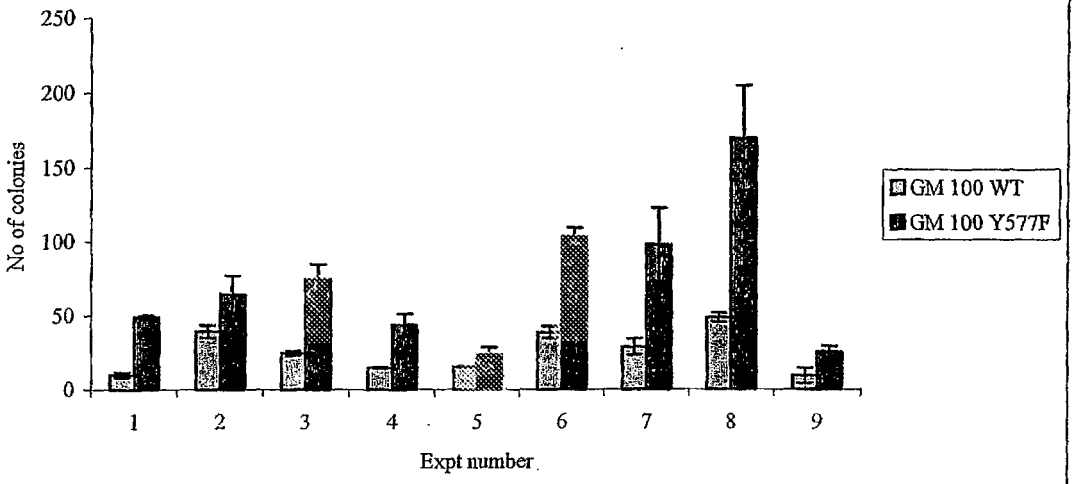

250
200
150
100
50
0
No of colonies
1
2
3
4
5
6
7
8
9
Expt number
GM 100 WT
GM 100 Y577F

Figure 2

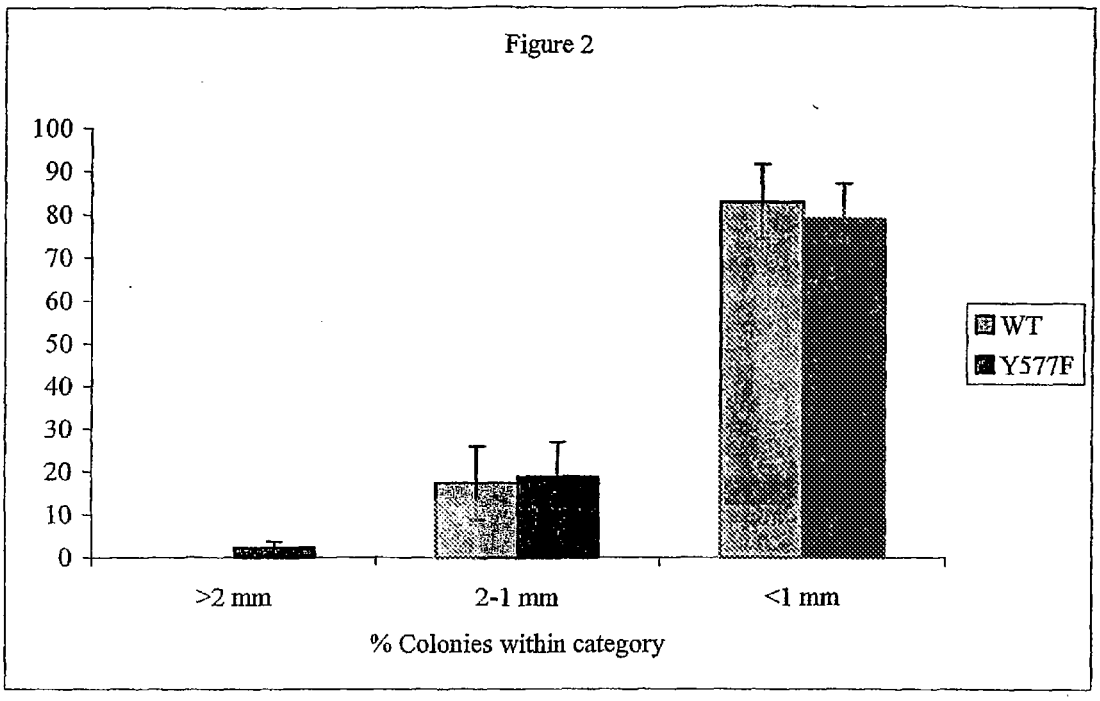

100
90
80
70
60
50
40
30
20
10
0
>2 mm
2-1 mm
<1 mm
% Colonies within category
WT
Y577F

Figure 3

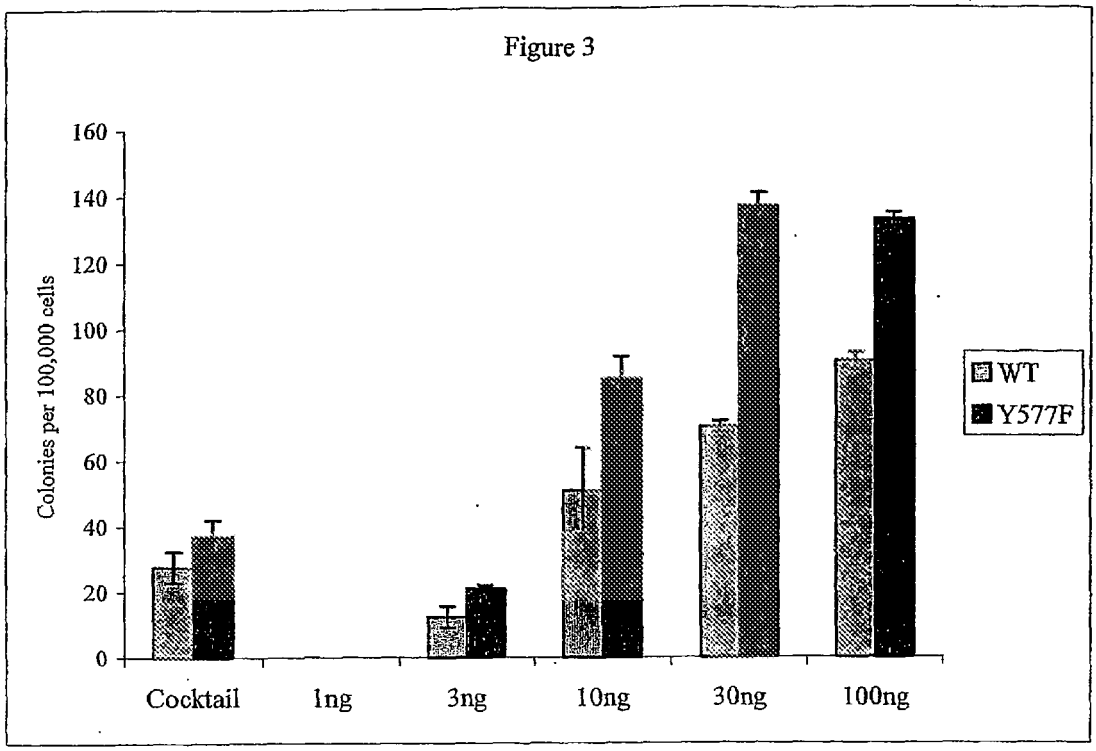

160
140
120
100
80
60
40
20
0
Colonies per 100,000 cells
Cocktail
1ng
3ng
10ng
30ng
100ng
WT
Y577F

Figure 4a

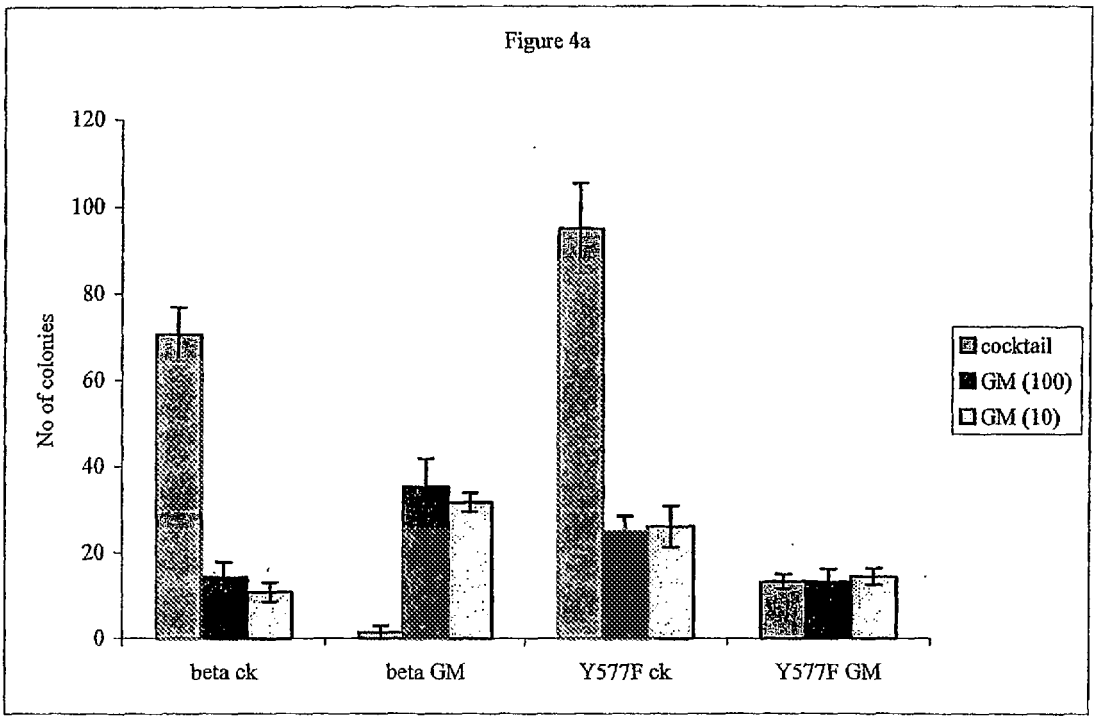
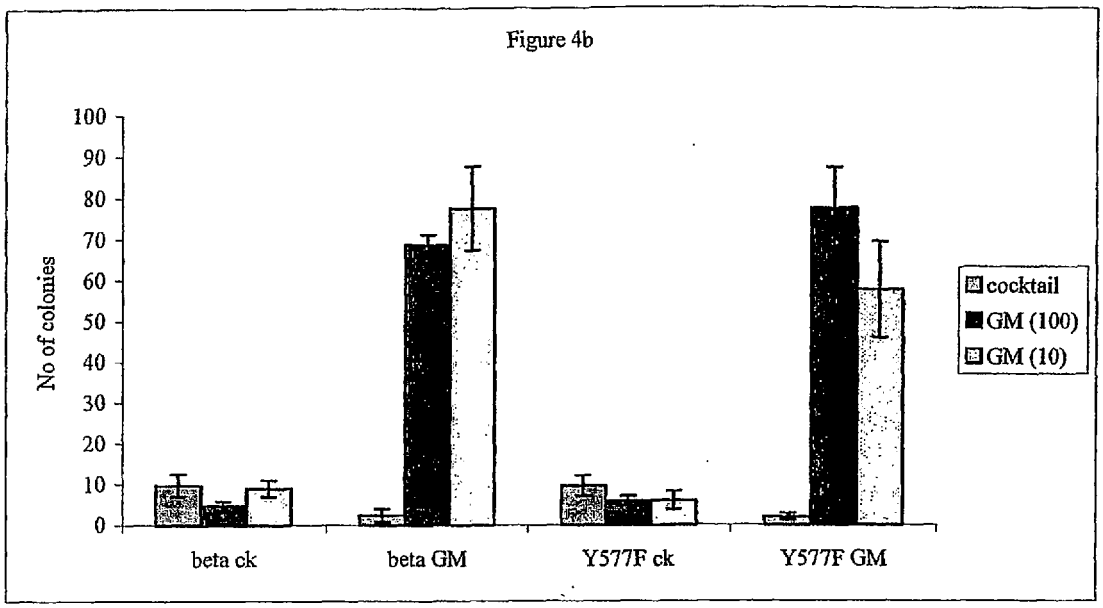

120
100
80
60
40
20
0
No of colonies
beta ck
beta GM
Y577F ck
Y577F GM
cocktail
GM (100)
GM (10)

Figure 4b


100
90
80
70
60
50
40
30
20
10
0
No of colonies
beta ck
beta GM
Y577F ck
Y577F GM
cocktail
GM (100)
GM (10)

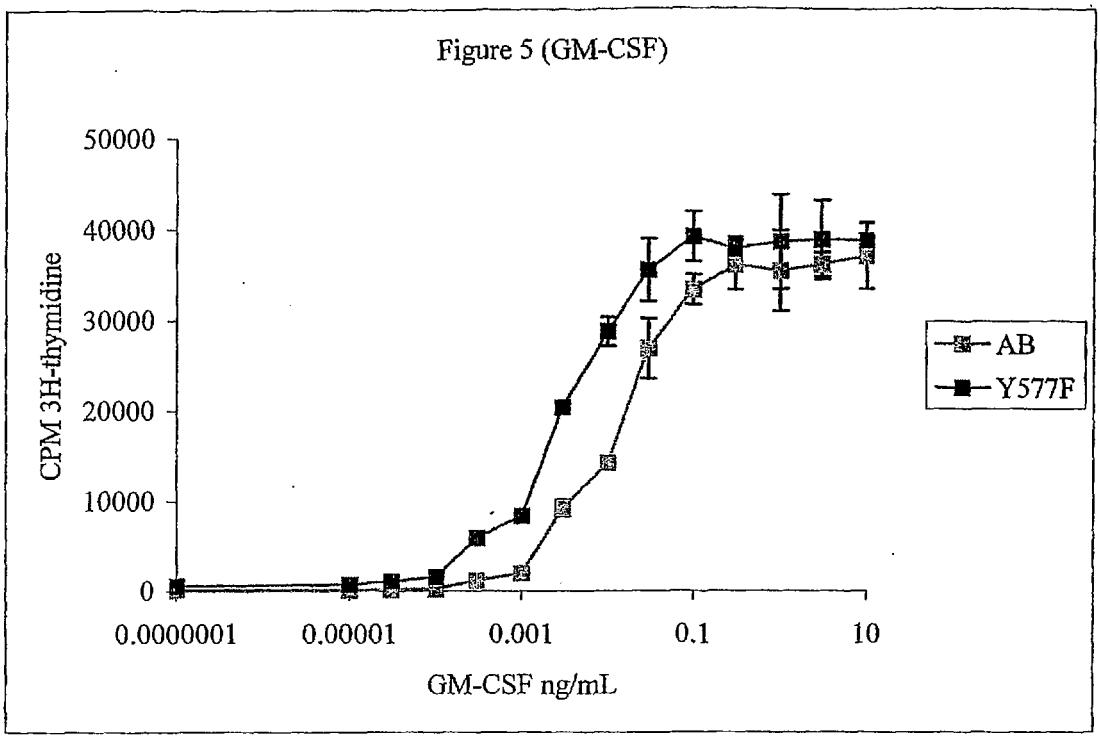


Figure 5 (GM-CSF)
50000
40000
30000
20000
10000
0
CPM 3H-thymidine
0.0000001
0.00001
0.001
0.1
10
GM-CSF ng/mL
AB
Y577F

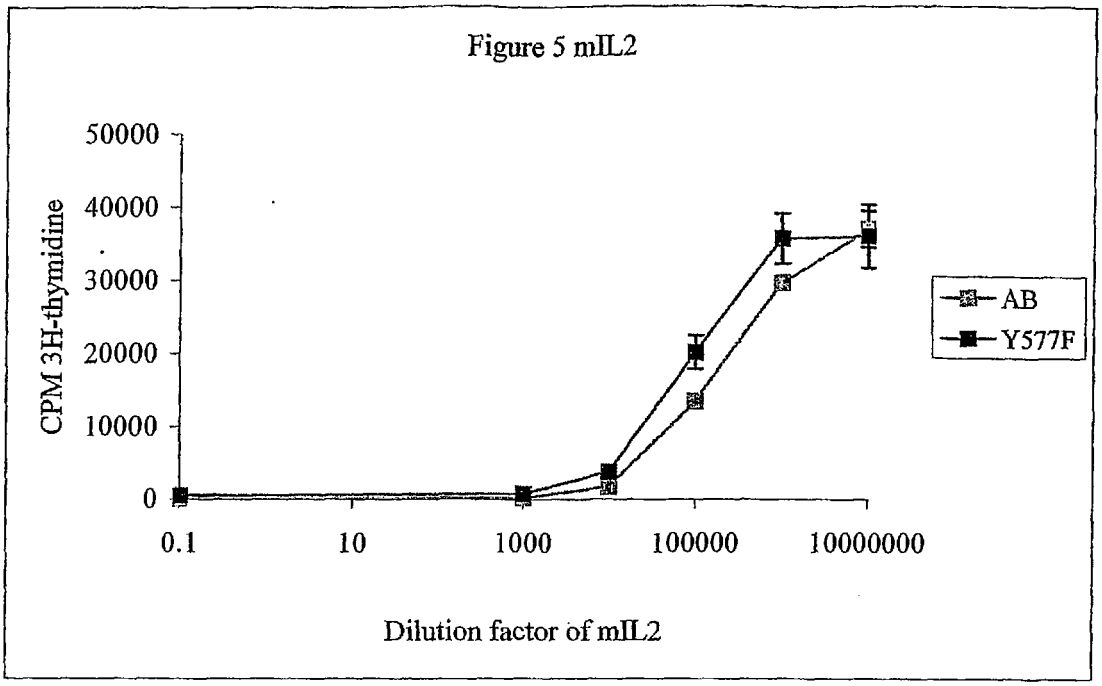


Figure 5 mIL2
50000
40000
30000
20000
10000
0
CPM 3H-thymidine
0.1
10
1000
100000
10000000
Dilution factor of mIL2
AB
Y577F

Figure 6

CYRB_HUMAN
CYTOKINE RECEPTOR COMMON BETA CHAIN PRECURSOR (CDW131 ANTIGEN)
Begin - 1, End - 897
Seq: CYRB_HUMAN Length: 897 Fri Nov 17 13:50:29 2000 Check: 148

```
   1  MVLAQGLLSM ALLALCWERS LAGAEETIPL QTLRCYNDYT SHITCRWADT
  51  QDAQRLVNVT LIRRVNEDLL EPVSCDLSDD MPWSACPHPR CVPRRCVIPC
 101  QSFVVTDVDY FSFQPDRPLG TRLTVTLTQH VQPPEPRDLQ ISTDQDHFLL
 151  TWSVALGSPQ SHWLSPGDLE FEVVYKRLQD SWEDAAILLS NTSQATLGPE
 201  HLMPSSTYVA RVRTRLAPGS RLSGRPSKWS PEVCWDSQPG DEAQPQNLEC
 251  FFDGAAVLSC SWEVRKEVAS SVSFGLFYKP SPDAGEEECS PVLREGLGSL
 301  HTRHHCQIPV PDPATHGQYI VSVQPRRAEK HIKSSVNIQM APPSLNVTKD
 351  GDSYSLRWET MKMRYEHIDH TFEIQYRKDT ATWKDSKTET LQNAHSMALP
 401  ALEPSTRYWA RVRVRTSRTG YNGIWSEWSE ARSWDTESVL PMWVLALIVI
 451  FLTIAVLLAL RFCGIYGYRL RRKWEEKIPN PSKSHLFQNG SAELWPPGSM
 501  SAFTSGSPPH QGPWGSRFPE LEGVFPVGFG DSEVSPLTIE DPKHVCDPPS
 551  GPDTTPAASD LPTEQPPSPQ PGPPAASHTP EKQASSFDFN GPYLGPPHSR
 601  SLPDILGQPE PPQEGGSQKS PPPGSLEYLC LPAGGQVQLV PLAQAMGPGQ
 651  AVEVERRPSQ GAAGSPSLES GGGPAPPALG PRVGGQDQKD SPVAIPMSSG
 701  DTEDPGVASG YVSSADLVFT PNSGASSVSL VPSLGLPSDQ TPSLCPGLAS
 751  GPPGAPGPVK SGFEGYVELP PIEGRSPRSP RNNPVPPEAK SPVLNPGERP
 801  ADVSPTSPQP EGLLVLQQVG DYCFLPGLGP GPLSLRSKPS SPGPGPEIKN
 851  LDQAFQVKKP PGQAVPQVPV IQLFKALKQQ DYLSLPPWEV NKPGEVC
```

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 731217	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT/AU2004/001480**	International filing date *(day/month/year)* 27 October 2004	Priority date *(day/month/year)* 27 October 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C07K 2/00; A61K 38/19, 38/20, C07K 7/06, 14/715, 14/71; A61P 35/00, 43/00

Applicant

MEDVET SCIENCE PTY LTD et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
25 May 2005	13 October 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **KATHERINE MOERMAN** Telephone No. (02) 6283 2714

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2004/001480

Box No. I Basis of the report

1. With regard to the **language,** this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1 (b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

☒ the international application as originally filed/furnished

☐ the description:

pages as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☐ the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☐ the drawings:

pages as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2004/001480

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos: **1-6 (in part)**

because:

☐ the said international application, or the said claims Nos.
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos.
are so unclear that no meaningful opinion could be formed *(specify)*:

☒ the claims, or said claims Nos. **1-6 (in part)**
are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. **1-6 (in part)**

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2004/001480

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **14-60**	**YES**
	Claims **1-13**	**NO**
Inventive step (IS)	Claims	**YES**
	Claims **1-60**	**NO**
Industrial applicability (IA)	Claims **1-60**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

The following documents, cited in the ISR, were considered for the purposes of this report:

D1 DATABASE NCBI (protein) Accession Number: AAA18171, 16 May 1994

D2 Bone H. and Welham M. J., Cellular Signalling, 2000, volume 12, pages 183-194

D3 Stomski F. C. et al., Blood, 1999, volume 94 (number 6), pages 1933-1942

D4 Guthridge M. A. et al., Stem Cells, 1998, volume 16 pages 301-313

D5 Itoh T. et al., The Journal of Biological Chemistry, 1996, volume 271 (number 13), pages 7587-7592

The invention of the present application is directed to the identification of a binding motif which contains the sequence NXXY. Claims 1-13 are directed to the binding motif. Claims 14-42 and 51-53 are directed to methods of modulating cellular activity. Claims 43-49 are directed to methods relating to the transplantation of cells. Claims 50, 52 and 53 are directed to methods for improving wound healing. Claims 54-57 and 60 are directed to the use of a substance which inhibits the activation of a tyrosine in a binding motif for the preparation of a medicament. Claims 58 and 59 are directed to methods for screening cell growth promoting compounds. While comments have been made regarding the dependency of some of the claims (Box VII), for the purposes of this opinion they have been considered as outlined above.

Claims 1-9, 12 and 13 are *prima facie* not novel in the light of the admitted prior art. Any protein, or peptide with the sequence NXXY is considered to anticipate claims to the binding motif (Box VIII). The sequences defined in Claim 4 are derived from known proteins (pages 12, 13). Consequently these proteins anticipate the claims.

D1 discloses the amino acid sequence of the common β chain of the GM-CSF, IL-3 and IL-5 receptors. Given that any protein, polypeptide or peptide with the sequence NXXY is considered to anticipate claims to the binding motif (Box VIII) Claims 1-13 are not novel in the light of D1. D1 does not disclose any information regarding methods of use of the protein. Therefore, Claims 14-60 are considered novel and inventive in the light of D1.

D2 discloses that the PTB domain of Shc interacts with the β chain at tyr577 and that NGPY is the binding sequence (section 3.6, 3.7 and discussion). Shc is known to be involved in the IL-3 signalling pathway and therefore, effects the cellular survival and proliferation. The mutation of tyr577 to phenylalanine, which results in the abolition of Shc activation is discussed in D2. D2 anticipates Claims 1-13 of the present application. D2 does not explicitly state the consequences of identifying the binding motif of Shc with the β chain. However, given that the signalling pathways and influence of IL-3 are known it would be obvious to the person skilled in the art that the manipulation of such signalling would result in the alteration of cellular activity as defined in the present claims. Therefore, Claims 14-60 lack an inventive step in the light of D2

Continued in supplemental box

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

a) Claims 1-6 are not fully supported by the description with respect to the term "binding motif". Independent Claims 1 and 2 define a binding motif capable of binding to a cytosolic protein and having the sequence NXXY.

Firstly, the use of the term "capable" indicates that the ability of the binding motif to bind to a cytosolic protein is not a limiting feature of the claim.

Secondly, protein-protein interactions depend not only on sequence (primary structure) but also secondary, tertiary and quaternary protein structure. Therefore, it appears that the claim is not directed to the four amino acids 'NXXY' in isolation but includes within its scope any protein or peptide that includes the sequence NXXY within its primary structure. The ability of one protein to bind to another protein is a property which is inherent to the proteins. Identifying and specifying the mechanism by which certain proteins interact does not render a claim to the proteins novel. Consequently, any protein or peptide which contains the sequence NXXY is considered to be highly relevant to the claims of the present application.

Thirdly, the description provides support only for the interaction of the motif found in the common β chain of the GM-CSF, IL-3 and IL-5 receptors at residues 574-577.

Therefore, Claims 1-6 are considered to lack the support of the description.

b) Claim 4 is not clear in scope or fully supported with respect to the phase "binding motif or equivalent thereof". There is no indication what an equivalent of the binding motif is within the claim. Furthermore, the definition provided within the description at page 11 for "functional equivalent or analogue thereof" is not clear in scope or fully supported as it defines potential equivalent proteins by the activity and function.

c) Claim 22 appears to be incorrectly appended to Claim 23. Claim 23 is directed to decreasing the phosphorylation of the Tyr by subjecting the cell to an antagonist, that is, indirect modification of the Tyr residue. Claim 22, which is appended to Claim 23 is directed to the direct modification of the binding motif by substituting the Tyr residue for Phe. It is perhaps more appropriate for Claim 22 to be appended to Claim 21 which is also directed to the direct modification of the binding motif.

d) Claims 38 and 39 appear to be incorrectly appended. These claims are dependent on Claims 34 and 35 respectively which in turn are appended to independent claims 14 and 15. The features defined in Claims 38 are also defined in Claim 16. The features defined in Claim 39 are also defined in Claim 17. Consequently, Claims 38 and 39 are redundant. However, Claims 38 and 39 follow independent Claim 37. The features defined in Claims 38 and 39 are not defined in the claims which are associated with independent Claim 37. Therefore, it seems more appropriate for Claims 38 and 39 to be appended to independent Claim 37.

Similarly, Claim 60 as appended to Claim 56 but follows independent Claim 58. The features defined in Claim 60 are also defined in Claim 57 resulting in Claim 60 being redundant. Therefore, it seems more appropriate for Claim 60 to be appended to Claim 58.

e) Claim 53 is redundant as the features which it defines are already defined in Claim 52 to which it is appended.

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of: V

D3 discloses a motif involving tyr577. Specifically, D3 discloses that the adaptor protein 14-3-3 ζ binds to residues 582-587 of the common β chain of the GN-CSF, IL-3 and IL-5 receptors. It is suggested that the proximity of tyr577 to the 14-3-3 binding site forms a distinct motif which is involved in specialized functions for the associated receptors (see discussion, paragraph 1). However, D3 does not disclose or suggest that the motif NXXY is capable of binding to a cytoplasmic protein and the amino acid residues upstream of tyr577 are not considered with respect to the motif disclosed. Therefore, Claims 1-60 are considered novel and inventive in the light of D3.

D4 discloses that the survival domain 'box 3' of the β chain (amino acid residues 570-626) is involved in signalling. D4 states "What motifs within this conserved domain are responsible for signalling, and how do they signal? Although this question addresses one of the most fundamental aspects of GM-CSF biology, the answer has remained elusive." (page 308, right column, first paragraph). D4 acknowledges the interaction between SHC and grb2 proteins with tyr577. However, D4 does not disclose or suggest that the motif NXXY is capable of binding to a cytoplasmic protein and the amino acid residues surrounding tyr577 are not considered in relation to protein binding. Therefore, Claims 1-60 are considered novel and inventive in the light of D4.

D5 discloses that Tyr577 is critical for the activation of Shc and mediates PTP1D phosphorylation. However, D5 does not disclose or suggest that the motif NXXY is capable of binding to a cytoplasmic protein and the amino acid residues surrounding tyr577 are not considered in relation to protein binding. Therefore, Claims 1-60 are considered novel and inventive in the light of D5.

The subject matter defined in the claims is considered to be industrially applicable.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001480

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 2/00; A61K 38/19, 38/20, C07K 7/06, 14/715, 14/71; A61P 35/00, 43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See electronic databases

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: Medline, CA, WPIDS, Biosis (GM-CSF, IL-3, IL-5, motif, beta, tyrosine, asparagine, tyr577, y577f)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE NCBI (protein) Accession Number: AAA18171, 16 May 1994 GM-CSF receptor beta chain. Sequence data	1-13
X	Bone H. and Welham M. J. "Shc associates with the IL-3 receptor β subunit, SHIP and Gab2 following IL-3 stimulation: Contribution of Shc PTB and SH2 domains", Cellular Signalling, 2000, volume 12, pages 183-194 Section 3.6, 3.7, page 192 paragraph 1	1-60
A	Stomski F. C. et al., "Identification of a 14-3-3 binding sequence in the common β chain of the granulocyte-macrophage colony-stimulating factor (GM-CSF), interlukin-3 (IL-3) and IL-5 receptors that is serine phosphorylated by GM-CSF", Blood, 1999, volume 94 (number 6), pages 1933-1942 Page 1939: discussion paragraph 1	1-60

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2004	27 JAN 2005

Name and mailing address of the ISA/AU

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001480

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Guthridge M. A. et al., "Mechanism of activation of the GM-CSF, IL-3 and IL-5 family of receptors", Stem Cells, 1998, volume 16 pages 301-313 Page 307-308 section titled 'Survival" and Figure 3	1-60
A	Itoh T. et al., "Granulocyte-macrophage colony-stimulating factor provokes RAS activation and transcription of c-*fos* through different modes of signalling" The Journal of Biological Chemistry, 1996, volume 271 (number 13), pages 7587-7592 Results and discussion	1-60

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001480

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **1-6**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The search has been limited substantially to the examples.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III *Observations where unity of invention is lacking (Continuation of item 3 of first sheet)*

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.